

The effect of fruit sap on the germination of four species of Tropical Plants

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Introduction

WHEN THE seeds of *Fagraea fragrans* Roxb. are extracted from their berries, washed thoroughly, dried and placed on damp filter paper, soil or any other media they do not germinate. Gardeners in Singapore obtain germination by squashing the whole berry and placing it in a pot of soil. It seems possible that the tissue which surrounds the seed in the berry contains some substance which is essential for the germination of these seeds. An investigation was made to determine more exactly the conditions necessary for germination and for their rapid growth under laboratory conditions. This led to investigation of three other plants with juicy fruits:—*Melastoma malabathricum* L, *Muntingia calabura* L. and *Duranta plumieri* Jacq.

The seed-coats of all four species of plants investigated are readily permeable to water. Except for *Duranta plumieri* Jacq. the seeds themselves are very small in size and counts of number of seeds germinated were made with a binocular microscope.

Investigation of the conditions necessary for germination.

1. *Fagraea fragrans* Roxb.

Fagraea fragrans Roxb. has beautiful scarlet berries which are about $\frac{1}{4}$ " in diameter. The trees in Singapore generally have two crops—one in September and one in January. The results given below were obtained using seed from the September crop of 1952 and the two crops in 1953. There was no significant difference between them. Great care was taken to select only ripe berries. Since the berries take nearly three months to mature there is a good number of intermediate stages. Ripe berries are bright scarlet in colour and are easily separable from the persistent calyx by a touch of the finger. Fallen berries were not used since it was impossible to be sure of the time they had lain on the ground and whether they had suffered any damage in their fall.

Gathered berries were squashed in some tap water with a pestle and mortar. Despite their small size the seeds were heavier than the water and berry tissue, and tended to form a bottom layer

which could be separated. The seeds were washed six or seven times with tap water and were filtered off, washed with distilled water, dried with filter paper and allowed to dry out for 24 hours. They were then completely free from sap and berry tissue.

In the above procedure the concentrated sap was retained and was diluted to a concentration 1 in 10, 1 in 100, 1 in 1,000. Fresh dry seeds were placed on filter paper in petri-dishes with distilled water and sap of each of the three concentrations above.

Other samples were placed with sap which had been filtered through Watman's No. 1 filter paper, and others with sap which had been both filtered and boiled. Table I gives the results.

TABLE I. PERCENTAGE GERMINATION OF SEEDS OF *FAGRAEA FRAGRANS*
(Samples contained approximately 100 seeds)

Water 0	Sap 1 in 1,000 90	Sap 1 in 100 92	Sap 1 in 10 93
Filtered Water 0	Filtered Sap 1 in 1,000 91	Filtered Sap 1 in 100 95	Filtered Sap 1 in 10 90
Boiled Water 0	Boiled Sap 1 in 1,000 95	Boiled Sap 1 in 100 90	Boiled Sap 1 in 10 96

From the above it is seen that there is no germination of washed, dried *Fagraea fragrans* seed in distilled water, but when the seed is provided with sap extract germination takes place readily. It is immaterial whether the sap is 1 in 1,000, 1 in 100, or 1 in 10 in concentration for they are all equally effective. The highest concentration (1 in 10) is rather syrupy and liable to fungal contamination during the experiment. The active principle for germination passes through ordinary filter paper since if any of the extracts are filtered they still are able to induce germination. Moreover, if the filtered extracts are boiled they still remain active. It seems that the active principle is soluble and thermostable in boiling water.

It may be stated here that tap water does not promote germination of these seeds and similar results are obtained throughout whether distilled or tap water is used.

If crushed or whole berries are planted in damp soil germination of the seed takes place in 12 to 14 days. It would seem that fallen fruit or fruit transported uneaten away from the tree by animals such as birds or bats would stand a good chance of germination provided the conditions were otherwise favourable.

The pH of the undiluted berry sap is in the region of 5.5–6.0. It was conjectured that a low pH might alone be the principle needed for germination of these seeds. Consequently a series of solutions of varying pH were made up in distilled water using M/5 solutions of sodium acetate and acetic acid. The pH values of these solutions varied by 0.5 unit intervals over a range of 3.5–7.0 units. Using washed dried seed it was found that germination did not occur at any of these pH's. Assuming that neither acetate or sodium ions are in themselves inhibitory to germination of these seeds it seems that the effect of berry sap is not merely a pH effect.

There have been reports (Barton 1940) that natural and synthetic auxins have a stimulatory effect on seed germination. The seed was therefore tested with a range of concentrations of 3-indole acetic acid in neutral solution from 1 part in 10^5 to 1 part in 10^{11} . No concentration was effective in promoting the germination of washed dried seeds. However, when other seeds were placed in the refrigerator at approximately 10°C and removed after a period of 1, 2, 7 or even 21 days, and then placed on filter paper with distilled water, germination of most of the seeds occurs after about 14 days at room temperature. The seeds were refrigerated in both a dry and wet condition and both samples germinated in subsequent tests.

For the purpose of sending seed samples abroad it was considered profitable to note the behaviour of washed dried seed which had been stored in a dry state for 10 days prior to germination tests. This seed was found not to germinate in distilled water alone. At the same time that the seed was extracted the berry sap was retained separately, diluted to 1 part in 100 and 1 part in 1,000 and autoclaved at 20 lb. for 30 minutes. This extract which was stored in conical flasks plugged with cotton wool remained free from contamination over the 10 day period. These extracts were added to the dry, 10 day old berry seed and a good percentage germination was obtained in both cases. But it may be somewhat inconvenient to send a quantity of autoclaved sap together with the dried seed. Germination can be more easily effected in 10 day old seed by placing it in the main body of a refrigerator (10°C) for a period of one or two days when germination occurs readily after 14 days at room temperature.

Berries were kept for 10 days attached to small branches and appeared but slightly shrivelled at the end of this period. The seed was extracted, washed and dried as before and the sap of these 10 day old berries retained for testing. The dried seeds would not germinate in distilled water. Moreover their own berry sap was.

not effective in promoting germination. The seeds were not however inviable and germinated if they were first in a refrigerator for two days, or supplied with berry sap from freshly gathered berries. It seems that the active principle which can promote germination in berry sap is lost if the berries are first plucked and stored for 10 days. As before stated the active component is not lost if the sap is extracted, autoclaved and stored for the same period.

Berries were dried in a steam oven and then stored for a 10 day period. The seed was then extracted, washed and dried as before and the sap retained separately. The seed would not germinate in distilled water alone, or if given their own berry sap (1 part in 100), or if given freshly prepared berry sap (1 part in 100) or if given autoclaved sap which had been prepared from berries 10 days previously, or if placed in a refrigerator prior to germination tests. The 100°C treatment had rendered the seed inviable and moreover had destroyed the active principle for germination in the berry sap since this was not effective in promoting the germination of freshly extracted seed. Similarly if the seed is autoclaved together with its own diluted sap, it is rendered inviable but in this case, as mentioned above, the sap extract remains active to viable seed.

II. *Muntingia calabura* L.

Like *Fagraea fragrans* the fruit of the *Muntingia calabura* is a berry containing small seeds. The berries have a somewhat larger diameter and are at first green, turning through pink to a deep red or black. If they are not eaten by children, birds or bats they decay becoming brown in colour. We did not know what stage would be best to obtain a good and easily repeatable germination, and experimented on seeds taken from green, red, and "decayed" berries with distilled water and with extracts of their own berry sap. The following results are the means of three complete experiments.

TABLE II. PERCENTAGE GERMINATION OF SEEDS OF MUNTINGIA CALABURA
(Seeds washed thoroughly to remove sap and adhering tissue)

Seeds from	With water	With Green Berry sap	With Red Berry sap	With "Decayed" Berry sap
Green Berry ..	24.0	8.3	10.5	3.9
Red Berry ..	23.3	9.5	6.3	3.1
"Decayed" Berry ..	61.5	3.3	4.7	0.0

From the above it is seen that the "decayed" fruit seed gives a much better percentage germination than that from green or red fruit. It is surprising that there is no difference in the percentage germination of the green and red berry seed. The sap from all three berries seems to be inhibitory. The dilution used in the above experiment was 1 in 100, but as a check seed from the "decayed" fruits was treated with sap diluted to 1 in 1,000. Inhibition still occurred.

Since the fruit of this tree is dispersed by fruit bats which eat the succulent portion and spatter pink masses of small yellow seeds (Corner 1952) the *Muntingia* sap may not have so great an inhibitory effect in nature.

III. *Melastoma malabathricum* L.

Melastoma malabathricum has a succulent fruit rather like a berry, but which opens when ripe by an irregular split. The unripe fruit is green and ripens to a red colour containing a purplish sap and a mass of small seeds. Three stages were used in the following experiments; green fruits, red but undehisced fruits and red dehisced fruits. All gave a good percentage germination in distilled water (the seeds were carefully washed to remove any adhering sap). They were also tested with suitably diluted sap extracts. Table III gives the results for seed taken from green fruit which had swollen to their maximum size. The percentage germination is somewhat variable but it can be seen that the sap has no significant effect on the germination of the seed.

TABLE III. THE PERCENTAGE GERMINATION OF SEED FROM GREEN, UNDEHISCED FRUIT WITH SAP EXTRACTS FROM ITS OWN FRUIT

Distilled Water	Sap 1 in 1,000	Sap 1 in 100	Sap 1 in 10
70.8	78.0	61.5	75.1
Filtered	71.8	77.2	82.4
Boiled and Filtered	60.0	73.7	76.9

In the case of the red, undehisced fruit the 1 in 10 sap has an inhibitory effect on germination. This effect is removed by boiling and filtering the extract.

TABLE IV. THE PERCENTAGE GERMINATION OF SEED FROM RED, UNDEHISCED FRUIT WITH SAP EXTRACTS FROM ITS OWN FRUIT

Distilled Water	Sap 1 in 1,000	Sap 1 in 100	Sap 1 in 10
72.7	69.2	72.2	34.6
Filtered	66.1	74.5	48.2
Boiled and Filtered	61.6	66.7	62.4

Similar results were obtained from fruits which had dehisced.

TABLE V. THE PERCENTAGE GERMINATION OF SEED FROM RED, DEHISCED FRUIT WITH EXTRACTS FROM ITS OWN FRUIT

Distilled Water	Sap 1 in 1,000	Sap 1 in 100	Sap 1 in 10
64.6	68.4	69.6	35.4
Filtered	54.2	63.2	25.2
Boiled and Filtered	66.4	65.6	58.2

IV. *Duranta plumieri* Jacq.

Duranta plumieri Jacq., has small orange berries containing a small number of hard seeds which are about 1/10 inch long. These seeds are considerably larger than any of the three types described above and occur in a much smaller number per fruit. Whereas the results given above are based on samples of 200-400 seeds, the following results are based on smaller samples each of only 20 seeds. They are included since *Duranta* is intermediate in behaviour between *Melastoma* (where dilute sap has virtually no effect on germination) and *Muntingia* (where the sap is definitely inhibitory).

TABLE VI. THE PERCENTAGE GERMINATION OF DURANTA SEEDS (Time of germination 20-25 days)

Distilled Water	Sap 1 in 1,000	Sap 1 in 100	Sap 1 in 10
20	20	10	0

Since the endocarp of *Duranta* is relatively hard it was thought germination might be increased by scarifying the coat. This was not so and the percentage germination of scarified seed was still only 20 per cent.

If the seed of *Duranta* is kept in the refrigerator at approximately 10°C for two days in either wet or dry condition it does not germinate on removing to room temperature. Unlike *Fagraea* the cold temperature does not increase the germination but inhibits it altogether.

V. Effect of alien sap on seed germination

Although *Duranta* sap inhibits the germination of *Duranta* seed it can promote the germination of the seed of *Fagraea fragrans*. The following are the results:—

TABLE VII. THE EFFECT OF DURANTA SAP ON FAGRAEA FRAGRANS SEEDS

With distilled water	With <i>Duranta</i> sap 1 in 1,000	With sap 1 in 100
0	38.3	22.7

It is to be noted that *Duranta* sap is not nearly as effective as the sap of *Fagraea* itself, which gives 90–96 per cent germination.

Fagraea fragrans sap is not always stimulatory since the germination of seeds of the split red fruits of *Melastoma malabathricum* is inhibited. The germination of seeds from the green and red unsplit fruits is not affected.

TABLE VIII. THE EFFECT OF FAGRAEA FRAGRANS SAP ON MELASTOMA SEEDS
(Results are means of three replications)

Distilled Water		Sap 1 in 100
Green fruit seed ..	70.8	70.8
Red fruit seed ..	72.7	64.4
Red dehisced fruit seed ..	64.6	36.7

The fruit saps of the four plants above no doubt vary greatly in composition and their effect is modified by individual properties of the four types of seed. It is unlikely that the stimulatory and

inhibitory effects described above are the result of any single substance or group of substances present in the fruit saps.

The presence of fruit sap may somewhat modify the percentage germination of seeds. Succulent fruits are generally attractive to animals which effect dispersal. The succulent portion may be eaten and the seeds ejected or swallowed. In the latter case they may still remain viable after passing through the alimentary canal. In *Muntingia calabura* L. the fruit sap is inhibitory to seed germination, and animals which eat the succulent portion and spit out the small seeds may perform a useful service in separating the seed from at least some of the inhibitory sap. In *Melastoma malabathricum* L. this is not important to subsequent germination since the fruit sap is not inhibitory. In the case of *Duranta plumieri* Jacq. the inhibitory effect of the sap is not as marked as in *Muntingia calabura* L. and removal of the fleshy part of the fruit by animals may not be so important. Incidentally, the berry of *Duranta plumieri* Jacq. is slightly poisonous to man. It does not however follow that it is poisonous to all animals.

The germination of the seed of *Fagraea fragrans* Roxb. is of some interest. It is generally believed that the fruit is eaten by fruit bats (Corner 1952) in which case the fruit sap which promotes germination of uneaten berries is presumably destroyed. The bats may however drop a sufficient number of uneaten berries to provide a sufficient means of dispersal. Alternatively seeds which have passed through the alimentary canal of a bat may be able to germinate in the absence of fruit sap due to physiological change caused by the fluids of the alimentary canal. We have no evidence on this point. Seeds which have not been eaten can only germinate if the berry seed falls or is dropped by a bat or bird onto favourable ground and is crushed to expose the seeds. If the seeds remain ungerminated for 10 days the fruit sap is no longer able to promote germination of the seed.

Concerning the cold temperature treatment of *Fagraea fragrans* seeds it is unlikely that this can be a factor in nature. If washed seeds are placed in an air-conditioned room at approximately 75°F for a day, they do not germinate on removal to room temperature. Temperatures do sometimes fall below this figure, especially in February which may coincide with the fall and dispersal of the January crop. There is no cold temperature period following the July crop of berries. Further experiments at a range of temperatures are necessary to determine the relationship between germination and cold temperature treatment.

References

- Barton, L. V. (1940) . . . "Some effects of treatment of seeds with growth substances on dormancy." Contributions to Boyce Thomson Institute II: 229-240.
- Corner, E. J. H. (1952) . . . "Wayside Trees of Malaya", Government Printing Office, Singapore. 2nd Edition.
- Henderson, M. R. . . . "Malayan Wild Flowers." Kelly & Walsh, Singapore.

Summary

1. Freshly washed and dried seeds of *Fagraea fragrans* Roxb. do not germinate on filter paper in distilled water.
2. If a diluted extract of the berry sap is added there is a 90-96 per cent germination in 12-14 days.
3. The "active principle" causing germination is filterable and thermostable at 100°C. It is not hydrogen ion concentration or an auxin.
4. Germination can also be effected on washed dried seed by refrigeration at 10°C at periods of 1-21 days prior to the germination test.
5. Ten day old seed will germinate if supplied with 10 day old berry sap which has been autoclaved and stored; or if refrigerated prior to testing.
6. The washed dried seed of *Muntingia calabura* L. give 61.5 per cent germination on filter paper in distilled water when fully ripe, but this germination is inhibited by sap extracts from its own fruit.
7. The germination of the seed of *Melastoma malabathricum* L. is unaffected by sap extracts from its own fruit.
8. The germination of the seed of *Duranta plumieri* Jacq. is inhibited by berry extracts at high concentrations but unaffected at lower concentrations.
9. From the effect of alien sap on seed germination it is likely that the active principle affecting the germination of these seeds is the same in all cases.